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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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2101	7590	07/05/2005	EXAMINER	
BROMBERG & SUNSTEIN LLP 125 SUMMER STREET BOSTON, MA 02110-1618			TON, THAIAN N	
			ART UNIT	PAPER NUMBER
			1632	
DATE MAILED: 07/05/2005				

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/995,452

Applicant(s)

BENVENISTY ET AL.

Examiner

Thaian N. Ton

Art Unit

1632

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 20 April 2005.
2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-9, 11-17, 36 and 59 is/are pending in the application.
4a) Of the above claim(s) _____ is/are withdrawn from consideration.
5) ☐ Claim(s) _____ is/are allowed.
6) ☒ Claim(s) 1-9, 11-17, 36 and 59 is/are rejected.
7) ☐ Claim(s) _____ is/are objected to.
8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
10) ☒ The drawing(s) filed on 01 March 2002 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Applicants' Amendment and Response, filed 4/20/05, has been considered and entered. Claims 1, 3, 11-17 and 36 have been amended. Claims 18-35 and 37-56 have been cancelled. Claim 59 has been added. Claims 1-9, 11-17, 36 and 59 are pending and under current examination.

The Benvenisty Declaration under 37 CFR §1.132, filed 4/20/05, has been considered and the Examiner's Response is found in the body of this Office action. Note: The Declaration is not considered proper because it has not been signed. However, for sake of compact prosecution, the Examiner has addressed the issues raised by the Declaration in this Office action. Applicants are requested to file the signed Declaration in response to this Office action.

Claim Objections

The prior objections to claims 3, 11, 12-17 are withdrawn in view of Applicants' amendments to the claims.

The prior objection to claims 57, 58, 49-56 are rendered moot in view of Applicants' cancellation of the claims.

Response to Declaration and Applicants' Response

The Benvenisty Declaration has been considered but is not found to be persuasive. The Declaration is provided to clarify the record concerning the

transfection of human embryonic stem cells relative to transfection of mouse and other animal cells. In particular, the Declaration seeks to provide evidence with regard to why it is not obvious to transfect DNA into human ES (hES) cells using transfection protocol other than electroporation, because electroporation was the transfection method of choice, until the instant invention, to transfect cells from various organisms (bacteria, mice, etc.); and further, to explain why achieving successful transfection of hES cells using protocols other than electroporation is unexpected and surprising; and to explain why those skilled in the art would not expect that the combination of the cited prior art, and modifications of mouse and other animal cell transfection protocols would be successful in achieving efficient transfection of hES cells. See p. 2 #2. The Declaration states that electroporation has been the method of choice for transfection from *E. coli* to mammalian systems, and point to Genetronics, a company which use electroporation as an efficient means to introduce genes into human cells. Further, the Declaration points to the Genetronics website as evidence that other methods of gene delivery, such as liposomes, cationic lipids, microinjection, or biolistic gun have inefficient gene transfer, and expression, or are inconvenient, invasive and costly. See #3, p. 3.

These arguments are not found to be persuasive. Applicants are arguing limitations that are not within the scope of the invention. The invention, put simply, is a method of transfection of hES cells. There is no yield requirement, percentage of cells or efficiency that must be acquired to practice the invention.

Thus, the art of record is maintained, because one, given the teachings of record, could practice the claimed invention with a reasonable expectation of success. It is not relevant whether other methods of transfection are more efficient, or inconvenient, invasive or costly. These are aspects that are not required within the claims; the claims only require that the cells are transfected. Thus, the transfection of a single hES cells would be sufficient to anticipate or make obvious the claimed invention. Applicants have clearly shown, on the record, that the transfection of hES cells by means, such as electroporation, will work, albeit with poor efficiency.

The Declaration teaches that prior to the claimed invention, no lab was able to stably transfect cells; that Applicants' expected that electroporation would be the protocol of choice for hES cells, but found this was not to be the case. Particularly, Applicants point to the fact that electroporation does not work well with transfection of hES cells, when compared with other cells. Applicants state that Zwaka (Exhibit C) show that hES cells have low transfection rate when using electroporation techniques. See #3-6, pp. 3-5 of the Response. Applicants point to Figure 1 of the specification to show how inefficient electroporation of hES cells is, and that the values in Figure 1 are inaccurate reflections of actual transfection rates, observed when using chemical transfection in the presence of the transfection reagents, as compared to transfection rates using electroporation. Applicants state that almost all of the hES cells were killed during electroporation and that the transfection rate depicted for electroporation in Figure 1 reflects a rate almost 10

times as high as many original cells. See #6-8, pp. 4-5 of the Response. Applicants point to Zwaka as further evidence that electroporation is inefficient to produce transfected hES cells; and that Eiges and Zwaka provide substantial evidence to show that mouse electroporation protocol did not work when the invention was submitted. Further, Applicants argue that while electroporation may be successful in transfection of murine ES cells, it is not successful for transfecting hES cells; particularly because there are substantial differences between mouse and human embryonic stem cells, and that electroporation did not work to transfect hES cells, which led for a different transfection methodology for hES cells, which is the claimed invention. See #9-11, pp. 5-7 of the Response.

These arguments have been considered, but are not persuasive. Applicants are arguing that electroporation as the only transfection method for hES cells. The art of record (particularly, Smith) contemplate other methods in order to transfect cells. Although electroporation is not an efficient method of transfecting hES cells, there is certainly a reasonable expectation of success to transfect hES cells, even if the resultant cells are not high in number. Smith contemplates other methods of transfection, which make obvious the claimed invention. Certainly electroporation would have been an option to transfect hES cells, but, in light of Smith's teachings, not the only method of transfection available to the skilled artisan. Thus, it is maintained that the art of record anticipates or makes obvious the claimed invention for reasons of record.

Applicants argue that with respect to transfection reagents and electroporation, those in the field would not perform electroporation in the presence of transfection reagents, and argue that a protocol that requires transfection in the presence of transfection, would be understood to be a chemical method of transfect, whereas electroporation would be considered a mechanical means of transfection, which would not require any additional transfection reagents to facility the entry to nucleic acids into the cell. See # 13, pp. 7-8. Finally, Applicants argue that those in the field trying to transfect hES cells would not infect them with an adenovirus because they are potentially dangerous, and could activate or introduce oncogenes, as adenoviral infection would not be compatible with the goal of obtaining stably-transfected hES cells for later therapeutic use. Thus, Applicants conclude that it would not be obvious, given the art of record, to use electroporation protocols to effectively transfect hES cells. See #14-15, pp. 7-8 of the Response.

Applicants' arguments have been considered, but are not found to be persuasive for reasons advanced above, and for reasons of record. In short, Smith *et al.* does not only contemplate electroporation techniques – they teach that transfection can be achieved by viral vectors, lipofection or electroporation. See col. 2, lines 61-64. The Examiner agrees that there would be no reason to use transfection reagents with electroporation, but Smith does not only teach utilizing electroporation. Furthermore, with regard to Applicants' arguments regarding using adenoviral vectors, Applicants are arguing limitations that are not in the

claims, and further, are intended uses of the claimed invention. This is outside the scope of what is instantly claimed. In particular, the claims are directed to methods of producing transfected cells. There is no requirement with regard to whether or not these hES cells would be safe for therapeutic uses, as the only requirement of the claims is the method steps of transfecting them. Accordingly, the Declaration is not deemed persuasive.

Applicants' Remarks and Arguments, which accompany the Declaration, have been considered, but are not found to be persuasive. With regard to the prior rejection of claim 36, under 35 USC §102, Applicants argue that the claims now recite, "a substantially pure transfected population of pluripotent hES cells". Applicants argue that because the claim limits the product to a population, and the population must be substantially pure, stably transfected, and pluripotent, Smith does not anticipate the claimed invention. See p. 7, #2 of the Response.

This is not persuasive. The metes and bounds of "substantially pure" are unclear. Furthermore, Smith teach that their method provides for isolation and/or enriching and/or selectively propagating animal stem cells (see col. 1-2, bridging). Thus, these selection or enrichment steps, as taught by Smith clearly result in what the skilled artisan would consider a substantially pure population of cells. In fact, Smith teach that a selectable marker, introduced into the stem cell, can be used to purify specific cells which express that marker. See col. 3, lines 60-65. Accordingly,

it is maintained that because Smith teach methods that result in transfected hES cells, they anticipate the claimed invention.

Applicants argue that, with regard to the art of Smith and the various references for dependent claims, Smith is not enabling for any transfection protocol, other than electroporation, because they only discuss using electroporation for transfection. Applicants then point to the Declaration as further evidence of this.

This is not persuasive, for reasons of record, and as stated above. Particularly, because Smith contemplates other methods of transfection, and further, that the claims do not require any particular amount or efficiency, thus to one of skill in the art, given the teachings of Smith, and the various other references (cited previously), the claimed invention would have been obvious, and could be practiced with a reasonable expectation of success. Applicants further summarize the Declaration, with regard to the inefficiency of electroporation in hES cells, and that electroporation unexpectedly killed most hES cells. See pp. 9-11 of the Response. All of these arguments have been addressed above in response to the Declaration. Applicants argue that even other chemical reagents shown in Figure 1 transfect hES cells better than electroporation, and thus, because hES cells are extremely fragile, and that electroporation itself kills most hES cells, results in low numbers of transfected cells, and that the combined art of Smith and, for example, Fasbender, do not provide motivation for combining and/or modifying the prior art, because there is no reasonable expectation of success. See p. 11, last ¶.

This is not persuasive. As stated above, Smith contemplates other methods of transfection, so the Examiner does not agree that they only are enabling for methods of electroporation. The fact that these other techniques are contemplated and then, combined with, for example, Fasbender, who specifically teach transfection using cationic molecules, provide the requisite motivation to render the claimed invention obvious, with a reasonable expectation of success. It is reiterated that the claims do not require any transfection efficiency, and thus, even one transfected cell renders the claims obvious. One of skill in the art could reasonably expect, given the art, which is replete in methods of transfection (including methods other than transfection), that, at least one hES cells would be transfected.

Applicants argue that, the claims 1 and 11, as instantly amended, state that the polynucleotide is introduced by transfection of either “at least one of the transfection reagents consisting of ...” and thus, this excludes both electroporation and adenovirus, because electroporation is not performed in the presence of a transfection reagent, and an adenovirus is not a transfection reagent. See p. 12, 1st ¶ of the Response.

This is not persuasive. As stated previously, the Examiner agrees that electroporation does not require the presence of other transfection reagents, however, the art of Smith is not only directed to electroporation, as noted above. Finally, the art of Fasbender, which utilizes adenoviruses to transfect cells does so

in the presence of cationic lipids. Thus, this fulfills the limitation that the transfection be in the presence of a cationic lipid.

Applicants argue that utilizing adenovirus in conjunction with transfection of hES cells is problematic because the resultant cells could not be used in therapeutic uses. Further, that there is no motivation to remove the adenovirus from the hES cells from the protocol. See p. 12–13. This argument has been addressed above; namely, that Applicants are arguing limitations that are not in the claims, and the intended use of the cells. These are outside the scope of the claims. There is no requirement for removal of the adenovirus in the instantly claimed invention.

Applicants argue that the various pieces of art which are used in combination of Smith do not render the claimed invention obvious, because there is no expectation of success, and the combination does not teach all of the elements of the claimed invention. See pp. 13-14 of the Response.

This is not persuasive. The cited art of record renders the claimed invention (with regard to the methods) obvious, because they teach each element when combined. One of skill in the art would perform such transfection techniques, with a reasonable expectation of success, because the claims do not require anything more than for an hES cells to be transfected.

Specification

The objection to the specification is withdrawn in view of Applicants' amendment.

Claim Rejections - 35 USC § 112

The prior rejections of claims 48-49, under 112, 2nd ¶, are rendered moot in view of Applicants' cancellation of the claims.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

The prior rejection of claims 36 under 35 U.S.C. 102(a) or 35 U.S.C. 102(e) as being anticipated by Smith *et al.* is *maintained* for reasons of record advanced in the prior Office action, mailed 11/17/04.

Smith anticipates the claimed invention because they teach methods of transfection of any mammalian embryonic stem cell, which include human ES cells. The method of producing these transfected cells does not depend on its method of

production. Accordingly, it is maintained that Smith anticipate the claimed invention. See also prior Office action, and *In re Best*.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-4, 6, 7, 11-16, 36, 59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.*, in view of Fasbender *et al.*, cited in the prior Office

actions. This rejection is maintained for reasons of record advanced in the prior Office actions.

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith teach that a selectable marker, introduced into the stem cell, can be used to purify specific cells which express that marker. See col. 3, lines 60-65. Smith do not specifically teach the formulation of the polynucleotide with a cationic non-lipid polymer transfection reagent for introduction into the stem cells.

However, prior to the time the claimed invention was made, Fasbender teach methods of transfecting various cell types utilizing complexes of cationic molecules

and adenovirus, which was found to enhance gene transfer *in vitro*. See Abstract. Fasbender teach COS-1, NIH-3T3 and 9L gliosarcoma cell cultures were used for the methods of transfection involving recombinant adenovirus vectors and various size poly-L-lysine hydrobromide polymers. The cells were subsequently infected and the uptake of the labeled adenovirus was assessed. See *Materials & Methods*. Fasbender teach that the expression of reporter genes was increased in the cultured cells when they were transfected with the combination of viral vector and cationic molecules.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claims 5 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* when taken with Fasbender as applied to claims 1-4, 6, 7, 11-16, 36, 59 above, and further in view of Myers. This rejection is maintained for reasons of record advanced in the prior Office actions.

Smith and Fasbender are described *supra*. They do not teach that the gene product encodes a fluorescent protein such as green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

However, prior to the time the claimed invention was made, Myers teaches that bioluminescent and chemiluminescent reactions are used as analytical tools in

various analytical applications, such as reporter gene studies. See p. 165, 2nd column, 1st ¶. Myers teaches that bioluminescent genes include the firefly luciferin and Renilla [see p. 165, 2nd column, lines 14-17 and #2]. Myers teaches that the gene for firefly luciferase has been cloned and is an effective reporter gene for studying transcriptional activity of cloned genomic sequences. See p. 168, #3.2.

Accordingly, in view of the combined teachings, it would have been obvious for one of skill in the art to utilize the methods of transfecting stem cells, as taught by Smith and Fasbender, and transfect a construct encoding a fluorescent protein, such as Renilla protein, or luciferase, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as it was well-known in the art to use such fluorescent proteins as reporter genes and various other assays, and as supported by Myers, "Bioluminescent reactions are used as analytical tools in protein and nucleic acid blotting, in nucleic acid sequencing and hybridization assays, and in reporter gene studies ... The main advantages to these reactions are their simplicity and analytical sensitivity." See p. 165, 2nd column, 1st ¶.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* in view of Fasbender *et al.* as applied to claims 1-4, 6, 7, 11-16, 36, 59 above, and further in view of Pascolo *et al.* is *maintained* for reasons of record.

As stated in the prior Office actions and preceding paragraphs, Smith and Fasbender provides teachings with regard to transfection of human ES cells that fulfill the limitations of the claims with regard to human ES cells. Furthermore, Pascolo provides the motivation to knock-out endogenous genes to analyze gene expression and, and that in generating the double knockout H-2D^b /mouse beta2 microglobulin, Pascolo states, "This should facilitate the study of HLA class I-restricted responses compared to classical transgenic mice. One might hope that the information gained with these animals will be of human relevance." See p. 2050, 2nd column, lines 4-7.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claims 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* in view of Fasbender *et al.* as applied to claims 1-4, 6, 7, 11-16, 36, 59 above, and further in view of the Gibco BRL catalog.

Smith and Fasbender are described *supra*. The Gibco catalog teaches LIPOFECTIN®, which is a liposomal formulation of a cationic lipid which is used to transfect a wide variety of cells, including human cells. See 1st ¶.

Accordingly, it is maintained that in view of the combined teachings of Smith, Fasbender and the Gibco BRL catalog, it would have been obvious for one of skill in the art to utilize the methods of transfecting human ES cells, as taught by Smith, by using a transfection reagent, such as LIPOFECTIN®, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to make such a modification, as it was an art-recognized goal to optimize transfection techniques of mammalian cells, and, as supported by the Gibco BRL catalog, that the LIPOFECTIN® reagent is a more efficient method of transfecting cells than calcium phosphate or DEAE-dextran transfection methods.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

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Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Thaian N. Ton whose telephone number is (571) 272-0736. The Examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the Examiner be unavailable, inquiries should be directed to Ram Shukla, SPE of Art Unit 1632, at (571) 272-0735. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the Official Fax at (571) 273-8300. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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